Sex Differences in Depressive- and Anxiety-like Behavior in Young Adult Npas4 Heterozygous Mice and Effects on GABAergic and Serotonergic Transmission

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Background and Introduction

• The GABAergic system is the main inhibitory system in the brain; deregulations of the GABAergic system have been implicated in increased susceptibility for depressive- and anxiety-like behaviors (Sibille et al, 2011).
•A recent study correlated increased anxiety in rats with decreased GABA receptor expression in the PFC (Wisłowska-Stanek et al, 2013).
• Npas4 is a transcription factor which is known to regulate GABAergic transmission, especially in the prefrontal cortex (PFC) and hippocampus (Coutellier et al, 2012) (Lin et al, 2008)
• The serotonergic (5-HT) system is highly connected with the GABAergic system, low levels of 5-HT transporter correlate with reduced Npas4 expression (Guidotti et al, 2012)
•5-HT receptors have been shown to modulate GABAergic neurons in the PFC and thus have an effect on depressive- and anxiety-like behaviors (Yan, 2002)

The purpose of this project is to identify a possible genetic mechanism conferring sex- and genotype-dependent susceptibilities to mood disorders through a mouse model. Determining whether abnormalities in the expression of Npas4 induces GABAergic and/or serotonergic deregulations and results in depressive- and anxiety-like behaviors may further confirm Npas4 as a major regulatory gene in mood disorder phenotypes.

Methods

Experimental Groups: (Behavioral and Molecular Assays)
- Npas4 heterozygous young adult males/females (HET-have 50% of normal Npas4 expression)
- Npas4 wild type young adult males/females (WT-have normal Npas4 expression)

Tests of anxiety-like behavior: (Fig 1a,b)

(1) Open Field Test (OFT)

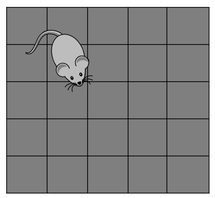

Fig 1a: 10 min. in square arena
- Increased time near walls indicate anxiety-like behavior

(2) Marble Burying Test (MBT)

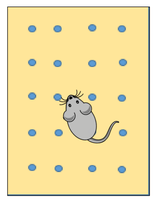

Fig 1b: 15 min. in rat cage with 20 marbles
- Fewer marbles buried indicates greater anxiety-like behavior

Test of depressive-like behavior: (Fig 2)

(1) Forced Swim Test (FST)

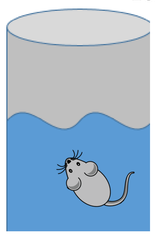

Fig 2: 6 min. in water in glass container
- Increased total immobility is a measure of depressive-like behavior
- Decreased time to first immobility also indicates depressive-like behavior

Behavioral Results

- Female HET mice display increased depressive-like behaviors compared to WT (Fig 3a,b)

Fig 3a: FST Total immobility (females)

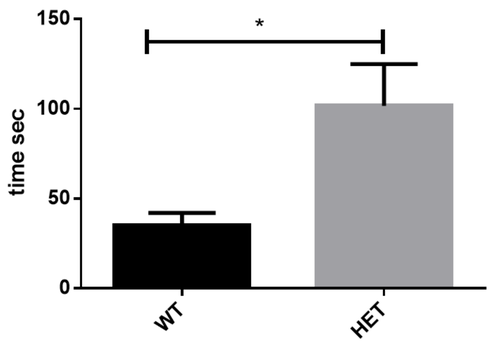

Fig 3a: Genotype effect, female HET mice show greater total immobility during FST indicating increased depressive-like behavior (*p<0.01).

Fig 3b: FST % Animals immobile during test

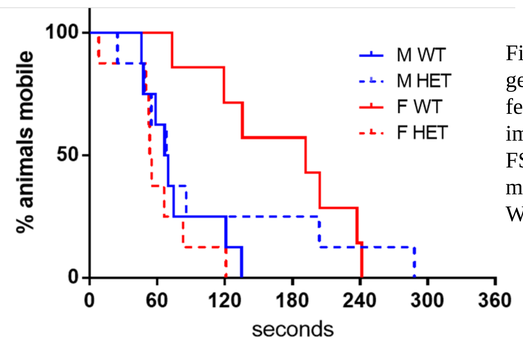

Fig 3b: Sex and genotype effect, more female HET mice were immobile earlier in FST than male HET, male WT, or female WT (p<0.0012).

- Male HET mice display increased anxiety-like behavior compared to WT (Fig 4a,b)

Fig 4a: OFT: Time in center (males)

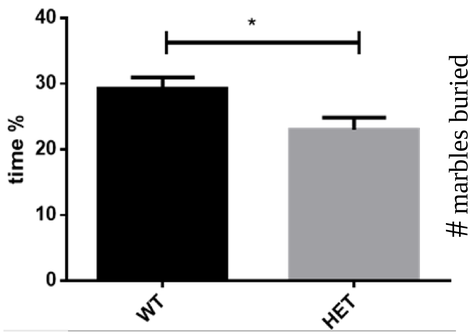

Fig 4a: Male HET mice spent less time in the center of the OFT arena than did male WT, showing increased anxiety-like behavior (*p<0.01).

Fig 4b: MBT: Marbles buried (males)

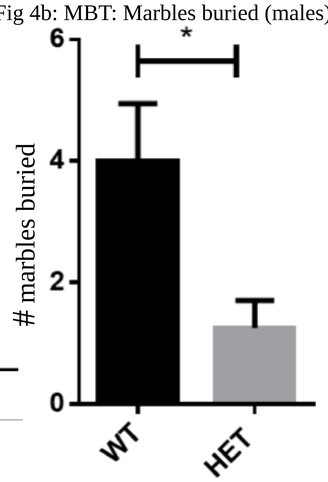

Fig 4b: Male HET mice buried less marble than male WT mice, indicating increased anxiety-like behavior (*p<0.01).

Molecular Results

- RT qPCR Receptor Expression: (Fig 5a,b)

Fig 5a: GABA-A α2 receptor expression

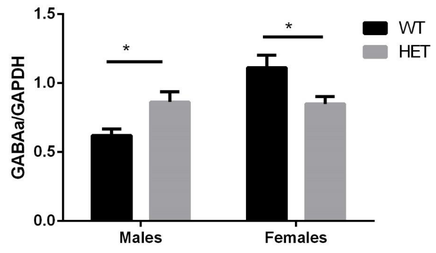

Fig 5a:
- Sex and genotype effect, GABA-A α2 receptor expression is lower in male WT mice and female HET mice (*p<0.01).
- This sex difference is correlated with anxiety-like behavior data
- Previous studies have found decreased GAD67 in male HET mice, therefore, compensatory effects resulting in up-regulation might have been shown in the present study.

Fig 5b: 5-HT1A receptor expression

Fig 5b: Genotype effect, lower expression of 5-HT1A in HET mice. Correlates with behavioral data in depressive-like behavior in female HET mice and anxiety-like behavior in male HET mice.

Conclusions

• Npas4 may regulate depressive-like behavior more in young adult females and anxiety-like behavior more in males.
• The genotype effect in 5-HT 1A receptor expression in Npas4 HET mice may underlie depressive phenotypes due to serotonergic dysfunction in Npas4 deregulation.
•The sex and genotype effect seen in GABA-A α2 receptor expression may underlie anxious phenotypes due to Npas4 deregulation of GABAergic systems.

Future Directions

Further investigations may better determine the genetic mechanisms in which GABAergic and serotonergic systems underlie susceptibilities to mood disorders.

References and Acknowledgements:

Coutellier L, et al, (2012) Npas4: A Neuronal Transcription Factor with a Key Role in Social and Cognitive Functions Relevant to Developmental Disorders. PLoS ONE 7(9): e46604. doi:10.1371/jmynal.pone.0046604

Guidotti, G., et al, (2012). Developmental Influence of the Serotonin Transporter on the Expression of *Npas4* and GABAergic Markers: Modulation by Antidepressant Treatment. *Neuropsychopharmacology*, *37*(3), 746–758. doi:10.1038/npp.2011.252

Lin, Y. et al, 2008. Activity-dependent regulation of inhibitory synapse development by Npas4. Nature. 455: 1198-1205.

Sibille, E, et al, (2011). GABA-related transcripts in the dorsolateral prefrontal cortex in mood disorders. *The International Journal of Neuropsychopharmacology*, *14*(6), 721–734. doi:10.1017/S14611457100016

Aleksandra Wisłowska-Stanek, et al, (2013). Changes in the brain expression of alpha-2 subunits of the GABA-A receptor after chronic restraint stress in low- and high-anxiety rats. *Behavioural Brain Research*, (253), 337-345. ISSN 0166-4328, http://dx.doi.org/10.1016/j.bbr.2013.07.042.

Yan Z. (2002). Regulation of GABAergic inhibition by serotonin signaling in prefrontal cortex.Mol. Neurobiol. 26, 203–216 10.1385/MN:26:2-3:20

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